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The analgesic effect of Tanacetum parthenium extract in formalin, acid acetic and hot plate test in mice

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Abstract

Tanacetum parthenium belongs to the Asteraceae family. This medicinal plant and other species have been widely used around the world since ancient times. It has been utilized to treat many diseases such as abdominal pain, indigestion and diabetes. In this experimental study, the analgesic effects of Tanacetum parthenium by formalin, hot plate and acetic acid test were evaluated in mice. In each test, the groups included distilled water (control), the extract with doses of 10, 20, 30, and 40mg/kg, ibuprofen 100 mg /kg, morphine 0.5 mg/kg, naloxane of 0.5 mg/kg and naloxane of 0.5 mg/kg and 10 and 40 mg/kg which was found to be the most effective dose of extract. Animals were injected with 0.9% acetic acid for visceral pain induction. 15 minutes after each injection, anti-nociceptive effects were recorded by counting the number of writhes during 30 minutes. In formalin test licking the hind-limb or jumping was recorded during 30 minutes. Mice were placed on hot plate with constant temperature of 48°C (5-minute intervals) for an hour. Results: The findings of this study showed that the ethanolic extract of Tanacetum parthenium had an analgesic effect ($P<0.05$). Injection of Naloxone blocked the analgesic effects of extract in formalin (61±3.15 to 111.5±56.4) and acid acetic test (6.2±3.01 to 19.6±5.1).Conclusion: This study verified the anti-nociceptive properties of Tanacetum parthenium compared to the control group and the anti-nociceptive activity of Tanacetum parthenium extract is due to interaction with opioid system. However, further studies are necessary to find the mechanisms effect of Tanacetum parthenium on pain.

Keywords

Morphine, Opioid, Visceral Pain, Tanacetum parthenium

1. Introduction

Damage to tissue causes pain and a reaction to remove the painful stimuli. In pathophysiology of pain there are very complex relationships between peripheral and central structures of the skin surface in cerebral cortex. The pain is a sensory, emotional and affective response. People have been using different forms of treatment in order to sedate pain for many years and plants have had an important role as sources of medicine. Some of the known pharmaceutical compounds such as aspirin, atropine, morphine and cocaine have been obtained from plants used as analgesic drugs [1]. The most common plant is Papaver somniferum from which morphine is obtained and is considered as the leader of opioid analgesics [2]. Due to the fact that the drugs had a wide range of side effects which were sometimes passed on to the next generations, studies on analgesic compounds have been done since 1960s.Also, it is believed that using total extract of the herb is more effective than a pure substance isolated from the herb [3].

In many clinical studies in the 1980s, ant nociceptive effects of Tanacetum parthenium (feverfew) from Asteraceae family have been considered [4] for the treatments of inflammation and migraine. In general, this herb blocks the release of serotonin which causes the onset of migraine pains [4, 5]. Also, it is used in traditional medicine in countries like Denmark for the treatment of epilepsy and it was found to have a great affinity for the place of benzodiazepines on GABA receptor [6]. All compounds of this herb are extracted by alcohol-ether, chloroform and water. The main ingredients are volatile oils.

In addition, this herb contains other compounds such as Tansyn, Terpene, and Sesquiterpene Lactone (including partenolid and sesquiterpenese (Alpha-Pinenes). It is anti-migraine, anti-inflammation and anti-rheumatism and has a bitter substance that is somehow toxic and is used in the exposure of intestinal parasites [4]. Czyz et al. revealed that the paratenoid, as one of the compounds of Tanacetum parthenium (TP), has anti-migraine effects together with anti-cancer effects[7,8]. Considering the excessive consumption of analgesic drugs in modern societies and side effects of these drugs, and also the limitations of their usage in special patients, more studies on alternative herbal and synthetic drugs are of great importance. Therefore, this study was conducted with the purpose of examining the analgesic effects and mechanism of TP extract using formalin, hot plate and acetic acid test.

2. Materials and Methods

Male mice(8-12 weeks and weighing 35-40 g) were housed individually in cages with an ambient temperature of 22°C - 25°C and a 12-h light/12-h dark cycle (lights on from 6:00 a.m– 6 p.m.). Animals were provided with water and food ad libitum. Experiments were carried out each day between 9:00 a.m. – 6 p.m. All experimental and animal-care procedures were performed according to international guidelines on the use of laboratory animals and approved by Shahrekord University of Medical Sciences Ethical Committee for Animal Research which is completely coincides with the ‘‘NIH Guide for the Care Use of Laboratory Animals’’.

Extraction: Collected samples of TP were prepared for the extraction after verification of genus and species using the existed valid identification keys. Then the aerial parts of the herb were separated and dried, away from light and high temperature conditions. Dried herbs were powdered and soaked in 70% ethyl alcohol for 2 days and the obtained extract was concentrated using rotary or (vacuum distillation). After evaporation of alcohol, the remaining substance was used to obtain the required concentration for the test [9]. In this study, 10 g was extracted from each 100 g of the dry powder. Herbarium code of this herb in the Shahrekord University of Medical Sciences is 210.

Acetic acid test: Generally, the abdominal writhing induced by intra-peritoneal (IP) injection of acetic acid as one of the standard tests was used to evaluate the effectiveness of new drugs in the treatment of visceral pain [10]. Writhing test is not only considered as a standard test for abdominal writhing but also is used in gastrointestinal ileus which can be evaluated through direct observation and counting abdominal writhing [11].

In this part, 90 mice were randomly divided into 9 groups. The groups included distilled water(control), the extract with doses of 10, 20, 30, and 40mg/kg, ibuprofen 100 mg /kg, morphine 0.5 mg/kg, naloxane of 0.5 mg/kg and naloxane of 0.5 mg/kg and 40 mg/kg of the extract which was found to be the most effective dose of it.60 mg/kg of %0.9 acetic acid were injected intraperitoneally (ip) to each mouse,15 minutes after the injection of extract [9]. Abdominal writhing was initiated after 10 minutes and it was recorded during 30 minutes. Distilled water, at the same amount of the extract (0.3 ml) was injected to 10 mice as the control group and after 15 minutes, the number of abdominal writhing was counted and recorded during 30 minutes [9]. Then, 20 mice in two groups received naloxane and naloxane + effective dose of the extract in order to examine the role of opioid receptors in the analgesic effects. In this study, 40 mg/kg of the extract has the most effective analgesic effect. Therefore, 0.5 mg/kg of naloxane, as the opioid receptor antagonist, with 40 mg/kg of the extract were subcutaneously injected with the interval of 15 minutes after the injection of extract (fig.1).

In sham group, only 0.5 mg/kg of naloxane was injected subcutaneously [9]. Also 20 mice were randomly divided into 2 groups. In the first group, 100 mg/kg ibuprofen and in the second group, 0.5 mg/kg morphine was injected (IP). At the end of each section of the study, the mice were excluded while observing the ethical principles. Finally, obtained data were analyzed using the statistical software of SPSS, Kruskal-Wallis test and Dunn post hoc tests.

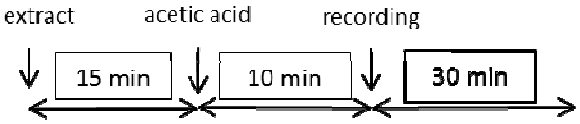

Fig 1. *The acetic acid test time line*

Formalin test: In this part of the study, 90 male mice were divided randomly into 9 groups like the acid acetic test groups. The first 5 minutes of the experiment was considered as the acute phase. Also, the time between 10 to 30 minutes was considered as the chronic phase. The time period to keep up and lick feet that injected Formalin was recorded. The extract was dissolved in distilled water, and then injected IP at doses of 10, 20, 30and 40 mg/kg.15 minutes after injection of the extract, 20 microliters of formalin 5.2 % were injected to foot. The reactions during30 minutes (every 5 minutes) were recorded. The most effective dose of extract was determined. Then, 15 minutes after injection of this dose, 0.5mg/kg naloxane as the opioid receptor antagonist was injected subcutaneously.

To compare the analgesic effect of the extract with the standard drug, ibuprofen and morphine was injected intraperitoneally, and after 15 minutes, 20 microliters of formalin 5.2 % was injected in foot and the responses were recorded during 30 minutes. In this study, the minimum dose of formalin was used. At the end of each section the mice were excluded from the study. Finally, data were analyzed with Kruskal-Wallis test and post hoc Dunn.

Hot plate test: In this stage of study, the groups were the same as the previous two tests. Order to reduce stress on the animals, before switching to the device, each mouse was put on the plate for 5 times. The extract in doses of 10, 20, 30, and 40 mg/kg was injected. During 60 minutes

(with 5-minute intervals) the mice were put on the hot plate with constant temperature of 48 ° C. The interval between putting the mice on the hot plate and the first response of the animals (including lifting the legs, feet licking, jumping of the animal, and vibrating along with its paw lifting) was considered as long time to reach pain threshold. In order to prevent the tissue damage, the test time was less than 30 seconds for each mouse. In addition, the experiment was done only once and the mouse was removed from the experiment. In the control group, distilled water was injected with the same amount of extract. In addition, in this part of the study, the effect of morphine (0.5 mg kg) and ibuprofen (100mg/kg IP) were evaluated in order to compare with the extract effect on the groups receiving it. To investigate the role of opioid receptors in the analgesic effects of the extract, the most effective dose of it was found (30 mg/Kg). Naloxane as an antagonist of opioid system was used. After injection of naloxane (0.5 mg/kg), the extract was injected subcutaneously. In the other control group, only naloxane was injected subcutaneously. At the end of the study, the mice were excluded with ethical principles. The data were analyzed with Kruskal-Wallis and dunnt post hoc.

3. Results

Acetic acid test: The repetitions of mice ratings in different study groups had significant differences according to Kruskal-Wallis test ($p<0.001$). However, Dunn post hoc test showed that during 30 minutes rating count, in different extracts groups, 40mg/kg extract had the most analgesic effect, ($p<0.001$) (Fig. 2).

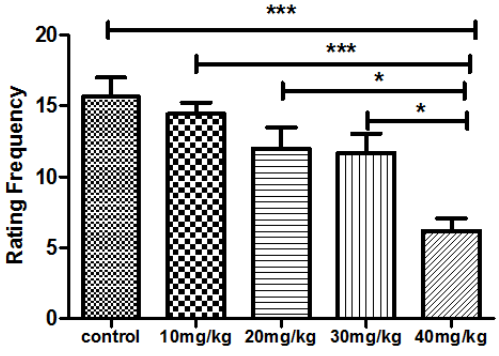

Fig 2. *The rating frequency (stretching the body) in different doses in acetic acid test*

This Fig Compared to the rating frequency (stretching the body) in different groups receiving the extract using acetic acid method and our data showed that 40mg/kg extract had the most analgesic effect, ($p<0.001$). *The most effective dose in reducing the frequency of rating ($p<0.001$).

40 mg/kg dose of the extract was significantly different from the other doses in the control group in reducing the frequency of rating ($p<0.001$).

However, the group receiving 40 mg/kg dose of the extract was not statistically different from the groups receiving morphine. But the group receiving ibuprofen was statistically different from the group receiving 10mg/kg of the extract. The anti-nociceptive effects was more in the group receiving 40 mg/kg of the extract ($p<0.001$) (Fig. 3).

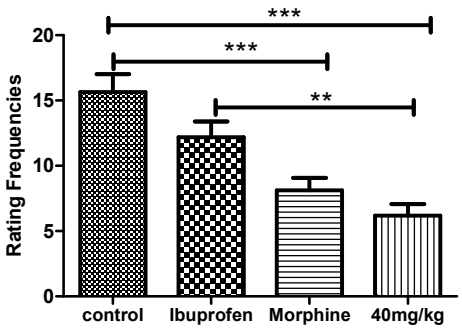

Fig 3. *The rating frequencies (stretching the body) in different groups in acetic acid test*

This Fig Compared to the rating frequencies (stretching the body) in different groups of standard and control (distilled water) using the acetic acid method. The group receiving 40 mg/kg dose of the extract was not statistically different from the groups receiving morphine. * There is a significant difference between the groups receiving ibuprofen Compared to 40 mg/kg dose of the extract.

There was a significant difference between the group receiving 40mg/kg of the extract and the group receiving ibuprofen ($p>0.001$).

The results of the study showed that the group receiving only naloxane has a significant difference with the groups receiving 40mg/kg of the extract + naloxane and the group receiving only 10mg/kg of the extract ($p<0.001$). Therefore, the group receiving 40 mg/kg of the extract showed more analgesic effect (Fig. 4).

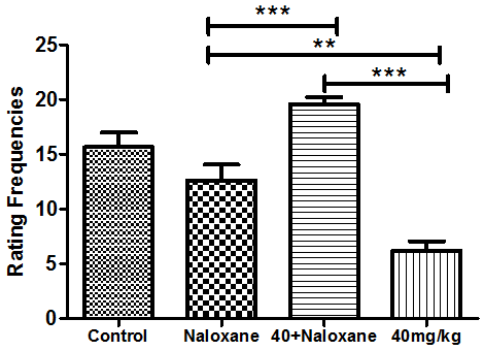

Fig 4. *The rating frequency (stretching the body) in the studied groups for evaluation of the opioid mechanism.*

This Fig Compared to the rating frequency (stretching the body) in the studied groups in evaluation of the opioid mechanism.* There is a significant difference between the group receiving naloxane and the group receiving 40 mg/kg of the extract + naloxane and the group receiving only 40mg/kg of the extract ($p<0.001$).

Hot plate test: The analysis of data using Kruskal-Wallis test showed that repetitions of mice ratings in the study groups, were significantly different ($p<0.001$).There was nosignificant difference between 30, 40and 20mg/kg doses of the extract, but the analgesic effect of30 mg/kg was

higherthan the other doses. In addition, there was a significant difference between the groups receiving distilled water and 30 and 40 mg/kg of the extracts (Fig.5) (P<0/01).

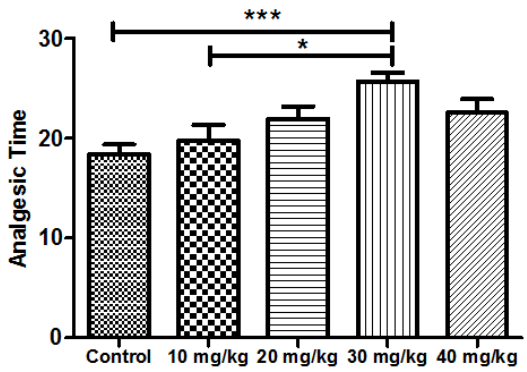

Fig 5. *The analgesic time in different doses of the extract and distilled water.*

This Fig Compared to the analgesic time in different doses of the extract and distilled water.There was no significant difference between 30, 40 and 20mg/kg doses of the extract, but the analgesic effect of 30 mg/kg was higher than the other doses. There was a significant difference between the groups receiving distilled water and 30 and 40 mg/kg of the extracts *(P<0.01).

The difference between the duration of analgesic effects of the most effective dose (30 mg/kg) and the standard treatment groups receiving morphine, and ibuprofen with distilled water was significant. However, there was no significant difference between morphine, ibuprofen and30 mg/kg extract (Fig.6).

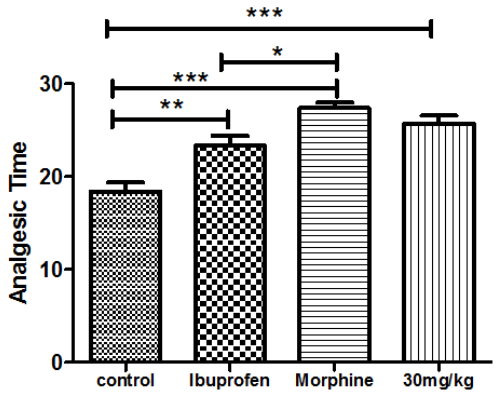

Fig 6. *The analgesic effect duration in 30mg/kg extract with the standard drugs.*

This Fig Compared to the analgesic effect duration in 30mg/kg extract with the standard drugs.The difference between the duration of analgesic effects of the most effective dose (30 mg/kg) and the standard treatment groups receiving morphine, and ibuprofen with distilled water was significant. *(p<0.001). There was no significant difference between morphine, ibuprofen and30 mg/kg extract.

Formalin Test:

In this test, the reaction time was different in the studied groups (p <0.05). The results showed that in the acute and chronic phase, the group receiving10 mg/kg extract had more analgesic effect and statistically significant differences with the other groups (p <0.01).

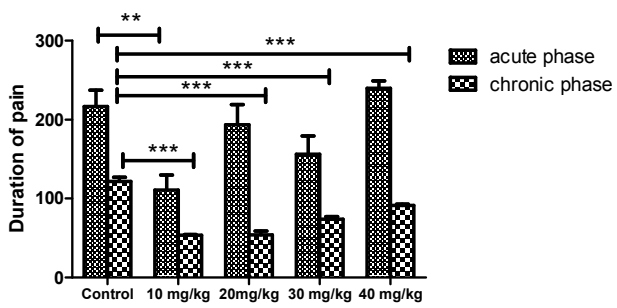

Fig 7. *The analgesic effects of different doses of the extract (mg/kg) and the control group in acute and chronic phase (seconds).*

This Fig Compared to the analgesic effects of different doses of the extract (mg/kg) and the control group in acute and chronic phase (seconds).the reaction time was different in the studied groups (p <0.05). The results showed that in the acute and chronic phase, the group receiving10 mg/kg extract had more analgesic effect and statistically significant differences with the other groups* (p <0.01)

The comparison of the effective dose of 10 mg/kg with the standard drugs showed significant difference between 10 mg/ kg of the extract, morphine and ibuprofen groups in acute phase (p <0.01). Furthermore, in chronic phase, difference between 10 mg/kg of the extract and ibuprofen group was significant (p <0.01)

In both acute and chronic phases, the analgesic effects of 10 mg/kg extract with naloxane were significantly less than only10 mg/kg extract (p <0.01), but there was no significant difference with morphine and ibuprofen (p>0.05) (Fig.8).

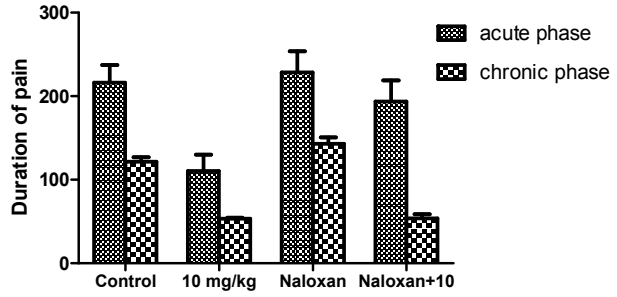

Fig 8. *The Analgesic Effects of the Extract in Presence of Naloxane*

Fig 8. This Fig Compared to the Analgesic Effects of the Extract in Presence of Naloxane. In both acute and chronic phases, the analgesic effects of 10 mg/kg extract with naloxane were significantly less than only 10 mg/kg extract (p<0.01), but there was no significant difference with morphine and ibuprofen (p>0.05)

4. Discussion

In this study, the analgesic effect of TP was studied in different pain test. The results showed that the group receiving 40, 30 and 10 mg/kg extract had more analgesic effects than the control groups. However, in acetic acid and formalin model, the group receiving effective dose of the extract had no significant difference with the group receiving morphine, but the analgesic effect of this dose

was better than ibuprofen. In hot plate model, this extract had no significant difference with morphine and ibuprofen. Naloxane reduced antinociceptive effects of the extract which indicates the possibility of analgesic effects of the extract using the opioid mechanism. Also, the results of this study showed that naloxane reduced the analgesic effects of the extract. It seems that the effect of this herb on pain relief is probably due to the opioid receptors. Because this effect has been greatly weakened in the groups receiving naloxane which are the antagonist of the opioid receptors (mu, delta, GABA) and naloxane injection has likely led to the blockage of opioid receptors [12].

In a study conducted by Iger, it was found that ethanol extract of the TP has great affinity for benzodiazepine sites on GABA receptor and inhibits and blocks the transmission of pain signals and reduces the pain [6]. Among the known and effective compounds of this herb, Paratinolid can be mentioned that, It is a peripheral analgesic compound because of inhibiting the production of thromboxane and leukotrienes [9].

Capasso evaluated the effect of aqueous extract of TP on arachidonic acid metabolism in the in-vitro conditions. In Capasso study, both oxygenase and lipooxygenase cycles were examined. The results showed that the metabolic products of both cycles are inhibited by the highest concentrations of the extract, but the lowest dose of the TP extract was effective on the arachidonic acid metabolism [13]. In this study, the dose of 40 mg/kg of the extract showed a better effect on reducing the pain which is consistent with Capasso, who stated that the highest concentration of the extract of the metabolic products inhibits both cycles. In this study which was conducted using different doses of the extract, abdominal writhing induced by acetic acid was reduced. In fact, the injection of the extract led to the dose-dependent reduction in the abdominal writhing. Presumably, the analgesic effect of TP on visceral pain could be achieved by inhibiting prostaglandins, similar to the non-steroidal anti-inflammatory drugs. The best known aspect of non-steroidal anti-inflammatory analgesic drugs (NSAIDs) is their effect on inflammation. Prostaglandin E2 stimulates pain receptors both directly and by enhancing their sensitivity to the other factors such as bradykinin (14).Therefore, NSAIDs may cause short-term and immediate recovery. NSAIDs analgesic effects are beyond their effect on inflammation and include wide central and peripheral actions. There are evidences of the effect of cyclooxygenase and prostaglandins in the central mechanisms of pain [14]. In the studies conducted by Christine et al. on the biological properties of flavonoids in the TP herb, the presence of 6-4 hydroxy-flavonol methyl ether in the extract of the leaf, flower and seeds were proved and anti-inflammatory properties of the most flavonoids called tannins were clearly specified in this herb [15]. Therefore, due to the presence of flavonoids in this herb, part of its analgesic effects is probably related to these compounds with the mentioned mechanism. Jain et al. also studied the analgesic effect of the extract of TP herb on inflammation and pain in rats and mice in the framework of acetic acid model and it was specified that the oral intake of the extract of this herb is associated with the analgesic and ani-inflammatory properties and the responses were dose-dependent(9). The results of this study about the pain reduction and the effect of the each doses of the extract are consistent with the results of the present study, but in the study conducted by Jain et al. the mechanism of the analgesic effect was not evaluated in accordance with the present study and no comparison was done with the standard drugs. So, in this regard, it is not comparable with the present study. Evaluation of the partinolid extracted from TP herb also showed that this compound has anti-inflammatory and analgesic properties

According to this study, TP herb has analgesic properties and it seems that steroids and especially flavonoids in the extract of this herb may prevent the formation of prostaglandins through cyclooxygenase inhibition in the inflamed tissue. Compounds of this herb, especially flavonoids, reduce pain and inflammation by activating numerous neural pathways. Therefore, it is needed to do further studies and recommend more analgesic mechanisms to be evaluated in future studies. Also, molecular tests can be considered to evaluate the effects of agonists and antagonists which may explain the analgesic effects of this herb.

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